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Differential effects of the cannabinoid receptor agonist, WIN 55,212-2, on lamina I and lamina V spinal trigeminal nucleus caudalis neurons

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Abstract

Direct application of cannabinoids to the medullary dorsal horn (MDH) inhibits lamina V nociceptive neurons. The present study compared the effect of the cannabinoid receptor agonist, WIN 55,212-2 (WIN-2) on the activity of lamina I and lamina V MDH neurons using extracellular single unit recording in anesthetized rats. Activity evoked by a contact thermode was measured before and after local application of WIN-2 (0.5–2.0 $\mu\text{g}/\mu\text{l}$) to the brainstem. Fast and slow heat ramps were used to differentiate between activity evoked primarily by A-delta and C primary afferent fibers, respectively. In lamina V neurons, WIN-2 produced a concentration dependent decrease in activity evoked by both fast and slow heat, reaching significance at 1.0 $\mu\text{g}/\mu\text{l}$. In lamina I neurons, WIN-2 administration inhibited slow heat evoked activity beginning at 1.0 $\mu\text{g}/\mu\text{l}$ but had no significant effect on fast heat evoked activity, even at the highest concentration (2.0 $\mu\text{g}/\mu\text{l}$). In separate experiments, the effect of intrathecal administration of WIN-2 to the MDH on head withdrawal latencies elicited by fast and slow heat ramps applied to the whisker pad was assessed in lightly anesthetized rats. Head withdrawal latencies elicited by slow but not fast heat stimulation were increased by WIN-2. Taken together, these results emphasize the importance of lamina I neurons in the control of a nociceptive heat-evoked reflex.

Introduction

Pain is a multifaceted response to tissue damaging or potentially damaging stimulation. The spinal and medullary dorsal horn (MDH), representing the first site of neuronal integration, receives nociceptive signals from primary afferent neurons and relays this information to brain and spinal cord regions involved in the various pain responses [56]. These components include, among others, behavioral reflexes, autonomic responses and pain sensation. Projection neurons with nociceptive receptive fields are located in both the most superficial layer, lamina I, and in deeper layers, including lamina V of the spinal and MDH [10,44,50]. Although differences have been noted in the projection targets and receptive field properties of lamina I and V neurons, the contribution of these neurons to the different components of the pain response has been largely a matter of debate [11,49,51].

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Cannabinoid receptor agonists inhibit nociceptive reflexes such as the tail flick through actions at the CB1 receptor [20,45]. Consistent with these findings, spinal and systemic administration of cannabinoid receptor agonists inhibits heat and mechanical stimulation-evoked activity of nociceptive neurons [19-21,30]. Most of these studies, however, have focused almost entirely on neurons located in the deep dorsal horn and failed to determine the projection status of recorded neurons. Furthermore, the ability to correlate cannabinoid-induced inhibition of withdrawal reflexes with suppression of neuronal activity is not possible since identical stimuli have not been used to evoke both reflex behavior and neuronal activity.

Stimulus parameters such as the rate in rise of temperature have important consequences on the type of primary afferent neurons that are activated. For example, slow heat ramps primarily activate C primary afferents, whereas fast heat ramps activate A-delta primary afferents [38, 39,41-43,61-63]. Utilizing this method, it has been demonstrated that systemic and spinal administration of morphine or a mu-opioid receptor agonist preferentially attenuates behavioral withdrawal responses to slow heat when compared to those elicited by fast heat applied to the hind paw [38,61]. These results are consistent with electrophysiological studies showing greater potency of morphine on C-fiber versus A-delta fiber evoked activity [8,28,29,35,58]. In order to gain insight into the function of MDH neurons located in superficial and deep laminae, the present study examined the effect of a cannabinoid receptor agonist on slow and fast heat evoked withdrawal reflexes and responses from superficial and deep MDH neurons.

Materials and Methods

All experiments were performed on male Sprague-Dawley rats (320-380g; Charles River). The present study was approved by the Committee on Animal Research at the University of New England, and animals were treated according to the policies and recommendations of the NIH guidelines for the handling and use of laboratory animals.

Single unit recordings

In the first set of experiments, the effect of the CB1/CB2 receptor agonist WIN 55,212-2 (WIN-2) on heat-evoked activity of units located in superficial and deep MDH was tested. Rats were anesthetized with continuous inhalation of 2-3% isoflurane mixed with oxygen for surgery. Following a tracheotomy, rats were artificially ventilated and end-tidal CO₂ was maintained at 3.5-4.5 percent. Body temperature was maintained at 37 °C by a feedback controlled thermal pad. The right external jugular was cannulated for administration of drugs, and the femoral artery was cannulated to monitor mean arterial blood pressure (MAP). Experiments were aborted if MAP could not be maintained above 85 mmHg. After placing animals in a stereotaxic holder, the dorsal brainstem was exposed and a C1 laminectomy performed [44]. A pool was made with skin flaps around the laminectomy, and the brainstem was kept moist with drug vehicle solution of 9:1, saline:emulphor (Alkamuls EL-620, Rhone-Poulenc, Cranbury, NJ) [19,40,46,47,60]. A PE-50 catheter was positioned at the level of C1 to empty the bath and infuse drug during recording. A hole was drilled in the parietal bone for insertion of a stimulating electrode into the contralateral thalamus or parabrachial area and electrophysiological recordings commenced at least 45 min after surgical preparation was complete.

Single unit electrophysiological recordings of MDH neurons located in superficial and deep laminae took place under 1.75-2.25 % isoflurane after paralysis with pancuronium (0.6 mg/kg/hr, i.v.). Activity was recorded with tungsten microelectrodes (impedance = 7 or 9 Meg ohms, 1000Hz, FHC, Bowdoinham, ME), and the signal was amplified, filtered and displayed on a digital oscilloscope after passing through a window discriminator (BAK, Germantown, MD) to monitor spike waveform. Activity from isolated units was acquired through a data

acquisition board interfaced with a PC and programmed in Labview (National Instruments, Austin, TX) [7].

Neurons were categorized as either wide-dynamic range (WDR) or nociceptive specific (NS) based on their responses to noxious and non-noxious mechanical stimulation of the receptive field [23,44]. WDR neurons responded to light brushing of the receptive field and increased their rate of activity in response to increasing pressure, whereas NS neurons did not respond to light brushing of the receptive field.

Thermal stimulation was applied to the center of the receptive field with a 5 X 5 mm contact thermode (Medoc, Minneapolis, MN). Two different heat ramps were used to preferentially activate A-delta or C-fiber primary afferents. As previously reported, rises in temperature of $< 2^{\circ}\text{C/s}$ activates C-fiber afferents, whereas heat ramps $> 2^{\circ}\text{C/s}$ results in activation of A-delta primary afferents [43,61,62]. Fast (3.4°C/s) and slow (1.0°C/s) heat ramps were used to increase temperatures from a 35°C holding temperature to a plateau of 52°C . The plateau temperature was maintained for 10 and 5 s for the fast and slow heat ramps, respectively. The sequence of fast and slow heat stimulation was applied with a three-minute interval between trials to avoid sensitization.

After two stable baseline trials of fast and slow heat stimulation, the bath was drained and filled with WIN-2 ($0.5 - 2.0\text{ }\mu\text{g}/\mu\text{l}$, Sigma-Aldrich, Saint Louis, MO), WIN-2 ($1\mu\text{g}/\mu\text{l}$) plus the CB1 receptor antagonist SR141716 (SR, $1\mu\text{g}/\mu\text{l}$, generous gift from NIDA), or vehicle. Approximately $100\text{ }\mu\text{l}$ of fluid was required to fill the bath covering the brainstem. Two additional trials of fast and slow heat were initiated at 10 and 25 min post-drug administration. In each animal, only one neuron and dose of drug were tested.

At the end of each experiment, contralateral projections to either the ventroposteromedial thalamic nucleus (ML 2.6, DV 5.0-7.0, AP -3.2 from Bregma) or parabrachial area (ML 2.6, DV 6.4-8.2, AP -8.8 from Bregma) were tested using a concentric bipolar stimulating electrode (FHC). Antidromic activation was identified by a discrete, all-or-none threshold, constant latency, the ability to follow high frequency stimulation, and demonstration of collision with orthodromic spikes [37,44].

Animals were perfused with saline followed by 10 % formalin after lesions of recording ($18\text{ }\mu\text{A}$, 20 s) and stimulation ($10\text{ }\mu\text{A}$, 10 s) sites. Frozen sections ($40\text{ }\mu$) were cut, mounted and stained with cresyl violet for histological verification.

Electrophysiology data analysis

Spontaneous activity was calculated as the average rate of activity for 10 s immediately prior to each stimulus. Heat evoked responses were determined by subtracting the spontaneous activity from the average rate of activity during the peak temperature. After-discharge was calculated as the average rate of activity for 10 s after termination of the heat stimulus minus the spontaneous activity. Neuronal activity was averaged for the two baseline control trials and two post-drug trials. Activity was converted to percent of control and compared between treatment groups using the Kruskal-Wallis test. This non-parametric alternative to the one-way analysis of variance (ANOVA) was chosen based on the significant difference in variance, as determined by the Levene test for the homogeneity of variances, combined with the differences in sample size. When overall significance was reached, post-hoc comparisons were performed using the Mann-Whitney U Test. The student t-test was used to compare changes in neuronal activity produced by WIN-2 ($1\mu\text{g}/\mu\text{l}$) with changes following co-administration of WIN-2 plus SR. In addition, the activation threshold upon application of the thermal stimulus was calculated according to the methods of McMullan and Lumb [42], in which the threshold was

defined as the point at which heat evoked activity became and remained elevated above the average spontaneous activity as measured for the 10 s prior to heat onset.

Head withdrawal latencies

In separate experiments, the effect of intrathecal administration of WIN-2 at the C1 level on the head withdrawal reflex elicited by thermal stimulation of the face was examined in lightly anesthetized rats. Five to seven days prior to behavioral testing, a laminectomy of the L5 vertebra was performed under isoflurane anesthesia. The dura was cut, and a soft silicone tube (VWR brand Select Silicone; outer diameter, 0.25 mm; VWR, USA) was backfilled with 0.9 % saline and inserted a length of 12 cm into the intrathecal space so the tip was just beyond C1 into cisterna magna. No animals demonstrated motor impairment during the recovery period.

Rats were lightly anesthetized with urethane (1.0 g/kg) at least 30 min prior to testing withdrawal latencies. This level of anesthesia remained stable throughout the test period and was adequate to eliminate spontaneous movements but still allow for withdrawal reflexes to light pinching of the forepaw [61]. Head withdrawal reflexes were elicited by noxious thermal stimulation applied to the center of the rat's shaved whisker pad region. Using a contact thermal probe, fast (3.4 °C/s) and slow (1.0 °C/s) heat ramps were applied to the face at three min intervals, and the latency to withdraw was recorded. The plateau temperature was 52 °C, and a cut-off period of 20 s was used to avoid tissue damage. After three stable baseline controls, intrathecal administration of WIN-2 (100-500 µg) or vehicle (9:1, saline:alkamuls) was delivered in a total volume of 10 µl followed by a 10 µl saline flush. Head withdrawal was evoked by two trials of fast and slow heat beginning 10 min after drug administration. After completion of the experiment, dissection around the C1 region was performed to verify the location of the catheter tip.

Behavioral data analysis

Latencies from the two post-drug trials and final two pre-drug trials were averaged for analysis. Head withdrawal latencies were converted into the percent maximal possible effect (%MPE) using the formula:

$$\text{Percent MPE} = [(\text{post-drug latency} - \text{pre-drug latency}) / (\text{cut-off time} - \text{pre-drug latency})] \times 100.$$

The effect of vehicle, WIN and WIN plus SR on withdrawal latencies was determined by a two-way ANOVA on the %MPE values. *Post hoc* comparisons were made using Newman-Keuls. All data are expressed as means $\pm$ SEM, and $P < 0.05$ was considered to be statistically significant.

Results

General properties

Recordings were made from 52 lamina I and 53 lamina V MDH neurons located between 0.5 and 3.0 mm caudal to obex. Of the lamina I neurons, all had either NS (20) or WDR (32) cutaneous receptive fields. Based on microdrive readings, the depth ranged from 0-212 µ with a mean of 39 µ and a median of 0 µ. Electrolytic lesions were recovered from 25 lamina I neurons, confirming the location of recording sites in superficial laminae (Fig 1A). In lamina V, 8 neurons had NS and 45 had WDR cutaneous receptive fields. Lamina V neurons were located deeper in the MDH, between 571-1611 µ with a mean of 1016 µ and a median of 1033 µ. The reconstruction of 20 histologically confirmed lamina V recording sites are shown in Fig. 1A.

Over 80 % of both lamina I and lamina V cells could be antidromically activated with stimulating electrodes positioned in either the PBA or thalamus. Lesioned stimulation sites in thalamus included both VPM and Po (Fig. 1B). Of 23 lamina I neurons and 28 lamina V neurons tested, 20 lamina I and 23 lamina V neurons could be antidromically activated from either the PBA or VP of the thalamus with an average latency of 1.9 ms (Fig. 1C).

Comparison of lamina I and lamina V neurons

In addition to the greater proportion of NS neurons characterized in lamina I (38%) compared to lamina V (15%), several differences between lamina I and V neurons were found prior to drug administration. Both fast and slow heat ramps evoked greater activity in lamina V neurons (Fig 2A, $p < 0.01$). Furthermore, lamina V neurons displayed a greater after-discharge to both slow and fast heat ramps compared to lamina I neurons (Fig 2B, $p < 0.01$). Although not statistically significant, lamina V neurons also tended to have greater spontaneous activity compared to lamina I neurons (1.7 ± 0.8 versus 0.5 ± 0.2 spikes/s, respectively). Finally, the receptive fields of lamina I neurons were generally restricted to one branch of the trigeminal nerve, whereas lamina V receptive fields often encompassed more than one trigeminal subdivision.

Effect of WIN55,212-2 on lamina I and V neurons

The baseline heat-evoked activity for all groups was not significantly different when compared with a 2-way ANOVA (Table 1). Data was converted to percent of control in order to compare the effect of drug treatment groups on fast and slow heat-evoked responses in lamina I and lamina V neurons. In lamina I neurons, bath application of WIN-2 (1.0 and 2.0 $\mu\text{g}/\mu\text{l}$) inhibited slow heat-evoked activity when compared to vehicle controls (Fig 3). By contrast, WIN-2 did not affect fast heat-evoked activity. Co-administration of WIN-2 with the CB1 antagonist SR prevented the inhibition of slow heat-evoked activity and administration of vehicle had no effect on heat-evoked activity.

Bath application of WIN-2 also inhibited neurons recorded in lamina V. However, unlike lamina I neurons, WIN-2 inhibited both fast and slow heat-evoked responses (Fig 4). The evoked activity of lamina V neurons to the fast heat ramp was inhibited at both the 1.0 and 2.0 $\mu\text{g}/\mu\text{l}$ doses of WIN-2, whereas a significant reduction in activity evoked by the slow heat ramp was reached only after application of the highest dose of WIN-2. This result contrasts with lamina I neurons, in which the slow heat-evoked response was more sensitive to inhibition by WIN-2. The inhibitory effect of WIN-2 on lamina V neurons was also prevented by co-administration of SR, and vehicle administration did not affect heat-evoked activity.

In both lamina I and lamina V neurons, the increase in threshold temperature to activate neurons did not reach statistical significance ($P > 0.05$, one-way ANOVA). However, the trends were consistent with the effects of WIN-2 on heat-evoked activity. After application of 1 $\mu\text{g}/\mu\text{l}$ WIN-2, activation thresholds in lamina I neurons increased an average of 2.5 ± 1.6 $^{\circ}\text{C}$ in response to slow heat ramp, whereas activation thresholds increased only 0.2 ± 0.8 $^{\circ}\text{C}$ in response to the fast heat ramp. In lamina V neurons, the same concentration of WIN-2 increased activation thresholds an average of 3.1 ± 1.8 $^{\circ}\text{C}$ during the fast heat trials and 2.4 ± 1.0 $^{\circ}\text{C}$ during the slow heat trials.

Effect of WIN 55,212-2 on withdrawal latencies

The effect of intrathecal administration of WIN-2 on the withdrawal latency of the rat's head was examined in 60 animals. In lightly anesthetized rats, fast and slow heat ramps were applied to the shaved whisker pad. Intrathecal injection of WIN-2 dose-dependently increased the withdrawal latency to the slow heat ramp, with significant inhibition beginning at the 200 μg dose (Fig 5). The antinociceptive effect of WIN-2 was prevented by co-administration of SR.

In contrast, withdrawal latencies to the fast heat increased only after the highest dose of WIN-2 (Fig. 5). Co-administration of SR (300 μ g) with WIN-2 (300 μ g) prevented WIN-2 induced antinociception. To control for the spread of drug rostral into brain regions involved in descending inhibition, we also tested paw withdrawal latencies following the C1 injection of 300 μ g WIN-2 in 8 rats. Although this dose of WIN-2 produced significant antinociception to the slow heat ramp when applied to the face, paw withdrawal latencies elicited by both slow and fast heat ramps remained unaffected (fast heat, 6.8 ± 0.5 versus 6.9 ± 0.3 ; slow heat, 16.9 ± 0.6 versus 17.7 ± 0.2 s).

Discussion

The present study utilized fast and slow heat ramps to evoke activity in lamina I and V MDH neurons. In lamina I cells, the direct application of WIN-2 to the MDH preferentially attenuated activity evoked by the slow heat ramp. In contrast, in lamina V cells WIN-2 inhibited activity evoked by both the fast and slow heat ramps. Furthermore, behavioral data demonstrated a more potent antinociceptive effect of intrathecal WIN-2 on withdrawal reflexes induced by the slow heat ramp. These effects of WIN-2 appear to have been restricted to the MDH, since paw withdrawal latencies remained unaffected by intrathecal administration to the C1/cisterna magna region.

Actions at the CB1 receptor were required for each of the aforementioned responses to WIN-2, demonstrated by the effectiveness of the CB1 receptor antagonist. Although WIN-2 can also bind to CB2 receptors, a CB2 receptor antagonist has been demonstrated to be ineffective in blocking the inhibitory effects of WIN 55,212-2 on MDH dura responsive neurons [1]. Those studies that indicate a role for CB2 receptors in the central actions of cannabinoids typically utilize more chronic pain models rather than acute reflexes, which may indicate an involvement of CB2 receptors on activated glial cells [53,54].

Previous investigators have employed low (<2.0 $^{\circ}$ C/s) and high (>2.0 $^{\circ}$ C/s) rates of heating to preferentially activate C and A-delta fibers, respectively [38,39,41-43,61-63]. Using this method in rats, capsaicin reduced withdrawal latencies elicited by slow but not fast rates of heating, consistent with the localization of TRPV1 receptors on C-fibers and the selective sensitization of these afferents by capsaicin [3,22,34,57,59]. Additionally, systemic or intrathecal administration of morphine has been shown to selectively increase withdrawal latencies to slow heating rates, which is consistent with the location of mu-opioid receptors on the terminals of C- fibers and preferential inhibition of C-fiber afferents by morphine [2,4,8, 14,15,27,28,35,58,61].

These previous studies selectively activated A- and C-fiber afferents with different rates of heating using both urethane and halothane anesthesia [38,39,41-43,61-63]. Our behavioral results were obtained with urethane anesthesia and electrophysiology experiments were conducted using isoflurane. Although no apparent differences have been reported for results obtained with urethane and halothane anesthesia, the correlation between behavioral studies and electrophysiological experiments should be treated with caution.

In results similar to those observed with morphine, the present study found that intrathecal administration of a cannabinoid receptor agonist preferentially increased withdrawal latencies elicited by the slow rate heat ramp. It might be expected that this result would indicate the selective expression of CB1 receptors on C-fiber primary afferent neurons. Unlike mu-opioid receptors, however, CB1 receptors are found primarily on myelinated primary afferent neurons of medium and large sized neurons in the dorsal root and trigeminal ganglia [6,16-18,52]. Furthermore, CB1 receptors do not tend to co-localize with calcitonin gene-related peptide, substance P or isolectin B₄, all markers of unmyelinated primary afferent neurons [6,52].

Together with the present results, these observations indicate that the differential effects of WIN-2 on A-delta and C-fiber evoked behavioral reflexes are due to actions on neurons located within the MDH rather than on the terminals of primary afferent neurons. Evidence for cannabinoid actions on inhibitory neurons within the MDH is provided by whole cell recordings from brainstem slices [25]. Although no direct postsynaptic actions were found, WIN-2 inhibited both GABAergic and glycinergic inputs onto superficial neurons in the MDH. These local circuitry effects of WIN-2 within the MDH may also explain the differences that were observed in the sensitivity of lamina I and lamina V neurons to WIN-2.

The specific neuronal pathways involved in head withdrawal reflexes have not been defined in detail. Cell bodies of motoneurons that control the neck musculature involved in head movement are located in the ventral horn of C1-3 [31-33]. The afferent component of this nociceptive reflex reaches the ventral horn motoneurons through multisynaptic connections within the brainstem and spinal cord that have largely been unexplored. One study that injected a retrograde tracer into the ventral horn of the upper cervical spinal cord indicated a substantial projection from lamina V in the contralateral lumbar and sacral spinal cord dorsal horn, with a lesser projection from lamina I at these levels [12]. Contralateral dorsal horn projections from both superficial and deep dorsal horn neurons in the upper cervical cord were also present but not described in this study (personal communication, E. Carstens). Additional insights into the reflex circuitry are provided by studies that have examined the anatomical pathways underlying both the tail and paw withdrawal reflexes [24,55]. In these studies, spinal cord dorsal horn neurons in both lamina I and lamina V were labeled upon tracking the transneuronal transport of the pseudorabies virus after its injection into muscles that control these reflexes.

While both lamina I and lamina V neurons appear to participate in nociceptive reflex circuits, the importance of lamina I neurons is illustrated in studies that have examined nociceptive reflexes and responses of dorsal horn lamina I and lamina V neurons in mice lacking the vanilloid receptor 1 (TRPV1) [9,13]. The TRPV1 receptor is activated by noxious heat, vanilloids, including capsaicin, and low pH [36]. Unexpectedly, TRPV1 deficient mice demonstrated similar behavioral responses to thermal stimulation between 43-48 °C [9]. Electrophysiology experiments in TRPV1-mice found that noxious thermal responses to temperatures as high as 49 °C were completely absent in lamina V neurons, but still present in some lamina I neurons [9,13]. It was concluded that the behavioral reflexes in mice lacking TRPV1 must be a result of the residual noxious thermal activity evoked in lamina I neurons.

The results of the present study are consistent with these findings. Correlating the effect of WIN-2 on both heat evoked neuronal activity and nociceptive reflexes implicates lamina I neurons in the control of noxious stimulation evoked reflexes, however a contribution from lamina V neurons is certainly not excluded. Whether WIN-2 is acting on primary afferent neurons co-expressing CB1 receptors and TRPV1 is unknown, but could account for the consistency between these findings. Some investigators have found considerable overlap between the distribution of CB1 receptors and TRPV1, while others have found little co-localization [5,52]. Through a CB1 receptor independent mechanism, however, WIN-2 has been shown to regulate the phosphorylation of TRPV1 in trigeminal neurons, which can desensitize and inhibit the channel [26,48].

In summary, intrathecal administration of WIN-2 inhibited noxious heat evoked reflexes evoked by slow but not fast heat ramps applied to the rat's whisker pad. Similar effects of WIN-2 were found on lamina I neurons, whereas both slow and fast heat evoked responses were inhibited in lamina V neurons. While these results seem to emphasize the importance of lamina I neurons in the control of a nociceptive heat-evoked reflex, lamina V neurons are also likely to play a role.

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Figure 1.

A) Location of lesion sites from lamina I and lamina V neurons recorded in the medullary dorsal horn (MDH). Numbers to the left represent approximate distance from obex (mm). Circles represent lamina I neurons and triangles represent lamina V neurons. **B)** Projection sites of MDH neurons in the contralateral ventroposteromedial thalamic nucleus (VPM, left) and parabrachial area (PBA, right) as identified by electrolytic lesions. Circles, stimulation sites for antidromically activated lamina I neurons; triangles, stimulation sites for antidromically activated lamina V neurons. Numbers represent distance from Bregma (mm). **C)** Example of an antidromically activated lamina I neuron by stimulation of the contralateral VPM. Neurons displayed a constant latency following a train of four stimulus pulses (200 Hz,

250 μ A), and collision with an orthodromic spike (bottom trace). Arrow indicates antidromic stimulus artifact. Closed circles indicate antidromically activated spikes and the open circle represents a failed attempt after collision with an orthodromic spike as marked by the asterisk. Po, posterior thalamic nucleus; VPL, ventroposterolateral thalamic nucleus; BC, brachium conjunctivum; CUN, cuneiform nucleus.

Figure 2.

Comparison of lamina V and lamina I nociceptive neurons in the MDH. **A)** Average activity evoked by fast and slow heat ramps in lamina V (filled bars) and lamina I (open bars) neurons. **B)** Average after-discharge in lamina V (filled bars) and I (open bars) neurons after activation with fast and slow heat ramps. * $P < 0.05$, ** $P < 0.01$.

Figure 3.

The effect of topical application of WIN-2 on heat evoked responses from lamina I neurons. Application of WIN-2 to the MDH dose-dependently attenuated the response to activity evoked by the slow heat ramp, whereas activity evoked by the fast heat ramp was not significantly affected even at the highest dose tested. Open and closed symbols represent activity by the slow and fast heat ramps, respectively. The inhibition of evoked activity was antagonized by co-administration of 1.0 μg/μl SR141716A (SR) with 1.0 μg/μl WIN-2 (triangles). * $P < 0.05$ compared with vehicle, ** $P < 0.01$ compared with vehicle, WIN-2 plus SR, and activity evoked by the fast heat ramp at the same dose of drug.

Figure 4.

The effect of topical application of WIN-2 on evoked responses of lamina V neurons. Application of WIN-2 to the MDH attenuated the fast and slow heat-evoked activity of lamina V neurons. Open and closed symbols represent activity by the slow and fast heat ramps, respectively. The inhibition of evoked activity was antagonized by co-administration of 1.0 μg/μl SR with 1.0 μg/μl WIN-2 (triangles). **P<0.01, response to fast heat compared with its vehicle control; ++ P<0.01, response of fast and slow heat compared with vehicle controls; ## P<0.01, response following WIN-2 plus SR compared with WIN-2 at the same dose.

Figure 5.

Intrathecal injections of WIN-2 to the Vc/C1 region increased head withdrawal latencies evoked by the slow heat ramp (open circles) applied to the face beginning at a dose of 200 μ g. Withdrawal latencies evoked by the fast heat ramp (closed circles) increased only after the highest dose of WIN-2. ** $P < 0.01$, latency to withdrawal from slow heat compared with vehicle; ## $P < 0.01$, latency to withdrawal from slow heat compared with fast heat.

Table 1

Average evoked activity (spikes/s) in control stimulation trials for each experimental group. The number of cells in each treatment group is indicated in parentheses.

	Lamina I		Lamina V	
	Fast heat	Slow heat	Fast heat	Slow heat
Vehicle	12.7 ± 3.3	8.4 ± 2.9 (5)	7.7 ± 1.9	7.9 ± 1.4 (7)
WIN-2 (0.5 µg/µl)	10.8 ± 3.9	7.0 ± 1.8 (6)	14.0 ± 4.4	12.1 ± 3.2 (8)
WIN-2 (1.0 µg/µl)	10.1 ± 3.1	9.0 ± 2.2 (14)	21.0 ± 5.0	19.5 ± 4.5 (15)
WIN-2 (2.0 µg/µl)	10.5 ± 4.1	10.3 ± 3.0 (8)	16.0 ± 5.0	21.7 ± 7.6 (9)
WIN-2 (1.0 µg/µl) + SR 141716	12.3 ± 2.6	13.2 ± 2.4 (12)	16.8 ± 5.4	14.9 ± 4.2 (14)